PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07H 21/02, 21/04, C12Q 1/68, G01N 33/50

A1

(11) International Publication Number: WO 95/00529

(43) International Publication Date: 5 January 1995 (05.01.95)

(21) International Application Number: PCT/US94/06897

(22) International Filing Date: 17 June 1994 (17.06.94)

(30) Priority Data:

08/079,677	18 June 1993 (18.06.93)	US
08/210,222	17 March 1994 (17.03.94)	US

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(71) Applicant: PHARMAGENICS, INC. [US/US]; 4 Pearl Court, Allendale, NJ 07401 (US).

(72) Inventors: COPPOLA, George, R.; 5 Holland Place, Hartsdale, NY 10530 (US). BEUTEL, Bruce, A.; Apartment 8206, 33 BonAire Circle, Suffern, NY 10901 (US). BERTELSEN, Arthur, H.; 215 Manor Road, Ridgewood, NJ 07405 (US).

(74) Agents: HERRON, Charles, J. et al.; Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068 (US).

(54) Title: INHIBITION OF INTERFERON-γ WITH OLIGONUCLEOTIDES

(57) Abstract

Oligonucleotides and analogs thereof that bind to and preferably modulate the activity of interferon-γ, and methods for their use, including therapeutic and diagnostic methods.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

INHIBITION OF INTERFERON-γ WITH OLIGONUCLEOTIDES

Interferon-γ (IFN-γ) is a multifunctional cytokine produced by, and secreted from, both T-lymphocytes and NK cells. The multiple forms of the cytokine all have an N-terminal pyroglutamic acid residue and up to two N-linked carbohydrates. The largest mature form of the cytokine consists of 143 amino acids but the carboxyl end of the protein is apparently heterogeneous due to post-translational proteolysis (Rinderkneckt et al., 1984, J. Biol. Chem. Vol. 259 p.6790).

IFN-γ exhibits antiviral activity as well as numerous immunomodulatory and anti-proliferative effects. Its effects on the immune system include influencing B-cell immunoglobulin class switching (Snapper et al., 1992, J. Exp. Med., *175*:1367), upregulating class I and class II MHC antigen expression (Buckmeier and Schreiber, 1985, Proc. Natl. Acad. Sci. USA, *82*:404; Bancroft et al., 1992, Immunol., 143:127), stimulating conversion of Th1 to Th2 lymphocytes (Kanagawa et al., 1993, Science, *262*:240), increasing macrophage-mediated killing of intracellular parasites (Portnoy, 1992, Curr. Opin. Immunol., *4*:20) and

promoting the production of IL-1, tumor necrosis factor, platelet activating factor, phosphoinositol kinase, 2-5A synthetase, indoleamine, 2,3 dioxygenase, hydrogen peroxide, pterin and nitric oxide (Nathan and Hibbs, 1991, Curr. Opin. Immunol., *3*:95; Grossberg et al., 1989, Experientia, *45*:508; Adolf, 1985, Oncology (suppl. 1), *42*:33; Samuel, 1991, Virology, 183:1; Billiau and Dijkmans, 1990, Biochem. Pharmacol. *40*:1433; Harris, et al., 1992, J. Biol. Chem. *265*:17868; Staeheli, et al., 1990, Adv. Virus Res. *38*:147). These various activities of IFN-γ are mediated by its binding to a specific cell-surface receptor protein found on a range of different cell types (Aguet et al., 1988, Cell, *55*:273; Valente et al., 1992, Eur. J. Immunol., *22*:2403).

Whereas many of the effects of IFN-γ on the immune system are beneficial to health and well-being, the activity of IFN-γ has also been shown to exacerbate certain pathological conditions. Immune system over-stimulation by IFN-γ has been implicated as a mediator of autoimmune reactions, as a contributing factor in the damage caused by septic shock and as contributing to the decline of immunocompetence in HIV infected individuals. Neutralizing antibodies directed against IFN-γ have been investigated as therapeutics in a number of disease model systems (Jacob et al., 1987, J. Exp. Med., 166:798; Umland et al.,1992, Clin. Immun. Immunopath., 63:66).

In a principal aspect this invention provides compounds that can bind to, and block the activity of, IFN-γ. In particular it has been discovered that certain oligonucleotides bind tightly to IFN-γ and block interaction with its specific receptor protein thereby neutralizing cytokine activity. The inhibitory oligonucleotides contain

discrete sequences that impart specific interaction with IFN-γ. Since the oligonucleotides of the instant invention bind tightly and specifically with IFN-γ it is a further object of the invention to provide diagnostic reagents and methods for detecting the presence of IFN-γ in test samples.

Figure 1

The binding of ^{32}P-labelled oligonucleotides (at tracer concentrations) to various concentrations of IFN-γ.

Figure 2

Elisa assay of IFN-γ receptor binding to plate-bound IFN-γ determining the inhibition of the interaction by added oligonucleotides.

Figure 3

Elisa assay of IFN-γ interaction with plate-bound IFN-γ receptor determining the inhibition of the interaction amounts of added oligonucleotides.

The present invention identifies oligonucleotides for binding with and preferably thereby modulating, inhibiting or enhancing, the activity or function of interferon-γ. The oligonucleotides are those which are capable of binding with reasonable affinity, preferably at a site which modulates th activity of the target molecule, IFN-γ. Such IFN-γ binding oligonucleotides can also be useful as diagnostic reagents for measuring levels of the cytokine.

Oligonucleotide compounds have been discovered that bind IFN-γ with high affinity and selectivity. Therefore, a principal aspect of the invention provides numerous oligonucleotides and portions of them that modulate, e.g.

inhibit, the activity of IFN-γ upon binding therewith. Oligonucleotides or poynucleotides are hereafter sometimes collectively referred to as "nucleic acid(s)". The nucleic acids so identified are not previously known to modulate IFN-γ activity and are preferably modified or unmodified nucleic acids containing one or more discrete, short (i.e. not more that about 20 bases) sequence elements. The nucleic acid can include flanking sequence at at least one of the 3' and 5' end(s).

More particularly, the preferred oligonucleotides of the invention are those that bind with IFN-γ at a Kd of not more than about 30 nM. Kd is determined by the method described in Riggs, et al., 1970, J. Mol. Biol., *48*:67-83.

In another aspect the invention provides nucleic acids or analogs thereof that bind to and affect the activity of IFN-γ and are preferably selected from the group consisting of SEQ ID NOS:1 to 29 including most particularly those oligonucleotides containing one or more sequences selected from the group consisting of SEQ ID NOS: 30 to 34.

Oligonucleotides of the present invention were effective in inhibiting IFN-γ activity when they included at least one of the following sequences: AAGUUG (SEQ ID NO:30); UGANGCUC (SEQ ID NO:31); UAAGUUGANGCUCG (SEQ ID NO:32); GCACCNC (SEQ ID NO:33); GCCACCCUCG (SEQ ID NO:34). Nucleic acid sequences are referred to using standard IUPAC abbreviation to specify the identity of the bases at individual positions of the oligomer.

A variety of fragments of individual oligonucleotides have been shown to bind tightly to IFN-γ. For example,

various subsequences of SEQ ID NO:3, identified in the SEQUENCE LISTING as SEQ ID NO:35 through SEQ ID NO:39, show a similar degree of binding to IFN-γ as the full length (98-base) oligonucleotide.

In addition to oligonucleotides having SEQ ID Nos. 1 through 39, the present invention further contemplates other oligonucleotides that bind to IFN-γ, and preferably inhibit the function thereof. Such additional oligonucleotides can be obtained readily by one of ordinary skill in the art using a variety of methods including, but not limited to, those described in Kinzler and Vogelstein, 1989, Nucleic Acids Research, Vol. 17, pgs. 3645-3652; Oliphant, Brandl and Struhl, 1989, Molecular and Cellular Biology, Vol. 9, pgs. 2944-2949; Kinzler and Vogelstein, Molecular and Cellular Biology, Vol. 10, pgs. 634-642; Thiesen and Bach, 1990, Nucleic Acids Research, Vol. 18, pgs. 3203-3209; Tuerk and Gold, 1990, Science, Vol. 249, pgs. 505-510; Ellington and Szostak, 1990, Nature, Vol. 346, pgs. 818-822; Gold and Tuerk, U.S. Patent No. 5,270,163 and Beutel et al., U.S. Patent Application No. 08/079,677.

The oligonucleotides of the invention may be in the form of a single strand, a double strand, a stem-loop, a bulged helix, a pseudoknot or a closed-circular structure.

In another aspect the invention provides a method of inhibiting IFN-γ function. The method comprises contacting IFN-γ with an effective amount of an oligonucleotide, or a molecule containing such an oligonucleotide, which inhibits IFN-γ function.

The term "inhibiting IFN-γ function" as used herein, means that the oligonucleotide prevents IFN-γ from being active, preferably by binding to IFN-γ. IFN-γ is a multifunctional cytokine produced by both T lymphocytes and NK cells. The effects of IFN-γ are mediated through binding to a specific transmembrane receptor, with the resultant activation and intracellular translocation of at least two known DNA-binding proteins.

In their several aspects, the methods of use of the nucleic acid sequences of the invention include inhibition of IFN-γ's antiviral activity, as well as its pleiotropic immunomodulatory and cell growth inhibitory effects.

Among the immunoregulatory effects of IFN-γ which can be modulated or inhibited are its influence on the class of antibody produced by B cells, its up-regulation of both class I and II MHC antigens, and its effect in increasing macrophage-mediated killing of intracellular parasites. All of these functions can be modulated or inhibited by the method of contacting IFN-γ with the nucleic acid sequences of the invention.

Another use in accordance with the invention is to inhibit the effect of IFN-γ to induce several genes, including HLA-B and HLA-DR, IP-10, P1 kinase, 2,5A-synthetase, and indoleamine 2,3-dioxygenase.

Another aspect of the invention provides the ability to inhibit the effect of IFN-γ to promote the production of interleukin-1 (IL-1), tumor necrosis factor (TNF), platelet activating factor, H_2O_2, and pterin.

Very interestingly, the invention provides a method to inhibit the propensity for IFN-γ to induce its own expression.

Yet another advantageous use provided by the inhibitory method of the invention is where an IFN-γ-inhibitory nucleic acid limits inappropriate IFN-γ-stimulated inflammatory responses in septic shock or rheumatoid arthritis.

Quinolinic acid, produced by the metabolic pathway catalyzed by 2,3-dioxygenase, damages neurons. Another of the many uses of the inhibitory method of the invention is the suppression of induction of this enzyme by an IFN-γ-inhibitory nucleic acid.

HIV-infected patients are less likely to develop AIDS if their CD4+ cells remain as Th1 as opposed to Th2. Since IFN-γ is implicated in the induction of a Th1 conversion to Th2 class, the IFN-γ-inhibitory nucleic acids of the invention are beneficial to HIV-infected individuals.

With respect to all of these aspects the term "inhibiting" refers to inhibiting one or more of the foregoing functions.

Nucleic acids that interact with proteins with high affinity are comprised of individual sequence elements that are highly conserved and other elements in which sequence is less well-conserved or even non-conserved. Both kinds of elements are important for the interaction of the nucleic acid and protein. For example, the sequence-specific elements might be the region that makes specific contacts with the protein whereas the less conserved regions might

serve a structural role in the presentation of the specific elements in the preferred configuration for protein binding. Furthermore, oligonucleotide polymers may be modified at many positions to impart new properties, such as resistance to nucleases, without destroying the desired characteristics of the oligonucleotide including its interaction with a target. For example, a ribozyme oligonucleotide generally consists of ribonucleotides yet many positions of the ribozyme oligonucleotide may be substituted with deoxyribonucleotides, making it more resistant to ribonucleases, without affecting its catalytic activity on its target RNA. Thus, the oligonucleotides of the present invention may be modified in a variety of ways to change certain characteristics, such as resistance to nucleases or ease of manufacture.

The term "oligonucleotide" as used herein means that the oligonucleotide may be a ribonucleic acid, i.e. an RNA oligonucleotide; a deoxyribonucleic acid, i.e. a DNA oligonucleotide; or a mixed ribonucleic/deoxyribonucleic acid; i.e., the oligonucleotide may include ribose or deoxyribose sugars, 2'-O-methyl ribose or other 2' substituted or conjugated sugars, or a mixture of such sugars. Alternatively, the oligonucleotide may include other 5-carbon or 6-carbon sugars, such as, for example, arabinose, xylose, glucose, galactose, or deoxy derivatives thereof or any mixture of sugars.

One or more of the phosphorus-containing moieties of the oligonucleotides of the present invention may be modified or unmodified. The phosphorus-containing moiety may be, for example, a phosphate, phosphonate, alkylphosphonate, aminoalkyl phosphonate, alkyl-thiophosphonate, phosphoramidate, phosphorodiamidate, phosphorothioate,

phosphorodithioate, phosphorothionate, phosphorothiolate, phosphoramidothiolate or phosphorimidate. It is to be understood, however, that the scope of the present inv ntion is not to be limited to any specific phosphorus moiety or moieties. Also, one or more phosphorus moieties may be modified with a cationic, anionic, or zwitterionic moiety. The oligonucleotides may also contain one or more backbone linkages which do not contain phosphorus, such as carbonates, carboxymethyl esters, acetamidates, carbamates, acetals, and the like. The oligonucleotides may also contain one or more backbone linkage of peptide nucleic acids. (Egholm, et al., J. Am. Chem. Soc., *114*:1895-1897 (1992)).

The oligonucleotides of the invention also include any natural or unnatural, substituted or unsubstituted, purine or pyrimidine base. Such purine and pyrimidine bases include, but are not limited to, natural purines and pyrimidines such as adenine, cytosine, thymine, guanine, uracil, or other purines and pyrimidines, or analogs thereof, such as isocytosine, 6-methyluracil, 4,6-di-hydroxypyrimidine, hypoxanthine, xanthine, 2,6-diaminopurine, 5-azacytosine, 5-methyl cystosine, 7-deaza-adenine, 7-deaza-guanine, and the like.

The oligonucleotides of the invention may be modified such that at least one nucleotide unit of the oligonucleotides may include a conjugate group. Such conjugate groups include, but are not limited to, (a) amino acids, including D-amino acids and L-amino acids; (b) peptides, polypeptides, and proteins; (c) dipeptide mimics; (d) sugars; (e) sugar phosphates; (f) neurotransmitters; (g) hormones; (h) poly (hydroxypropylmethacrylamide); (i) polyethylene imine; (j) dextrans; (k) polymaleic anhydride;

(l) cyclodextrins; (m) starches; (n) steroids, including sterols such as, but not limited to, cholesterol; (o) acridine; (p) vitamins; and (q) polyalkylene glycols, such as polyethylene glycol. Such moieties may make the oligonucleotides more resistant to degradation in cells and in the circulation, and/or make the oligonucleotides more permeable to cells. The conjugate moiety may be attached to the 3' terminal nucleotide unit and/or the 5' terminal nucleotide unit and/or to an internal nucleotide unit(s), or conjugate moieties may be attached to two or more nucleotid units at the 3' end and/or the 5' end of the oligonucleotide. In one embodiment, substituted nucleotide units may alternate with unsubstituted nucleotide units. In another embodiment, all of the nucleotide units are substituted with a conjugate moiety.

The conjugate moiety may be attached to the oligonucleotide at the purine or pyrimidine base, at the phosphate group, or to the sugar. When the conjugate moiety is attached to the base, it is preferably attached at certain positions of the base, depending upon the base to which the moiety is attached. When the moiety is attached to adenine, it may be attached at the C2, N6, or C8 positions. When the moiety is attached to guanine, it may be attached at the N2 or C8 positions. When the moiety is attached to cytosine, it may be attached at the C5 or N4 positions. When the moiety is attached to thymine or uracil, it may be attached at the C5 position.

In one embodiment, the oligonucleotide includes from about 5 to about 100 nucleotide units, preferably from about 8 to about 60 nucleotide units.

In yet another embodiment, th oligonucleotide represents a portion of a larger molecule which contains non-oligonucleotide components, such as, for example, peptides or proteins, or simple carbohydrates, and lipids.

The oligonucleotides of the present invention may be in the form of a single strand, a double strand, a stem-loop structure, a pseudoknot, or a closed, circular structure. In one embodiment, the ends of the oligonucleotide may be bridged by non-nucleotide moieties. Examples of non-nucleotide bridging moieties include, but are not limited to, those having the following structural formula:

T_1-R-T_2, where T_1 and T_2 are each independently attached to a nucleotide phosphate moiety or a hydroxyl moiety. R is selected from the group consisting of (a) saturated and unsaturated hydrocarbons; (b) polyalkylene glycols; (c) polypeptides; (d) thiohydrocarbons; (e) polyalkylamines; (f) polyalkylene thioglycols; (g) polyamides; (h) disubstituted monocyclic or polycyclic aromatic hydrocarbons; (i) intercalating agents; (j) monosaccharides; and (k) oligosaccharides; or mixtures thereof. In one embodiment, the non-nucleotide bridging moiety may be a polyalkylene glycol such as polyethylene glycol.

In another embodiment, one or more of the non-nucleotide moieties R may be substituted for one or more of the nucleotide units in the target protein binding sequences, as hereinabove mentioned.

The oligonucleotides of the present invention may be synthesized by a variety of accepted means known to those skilled in the art. For example, the oligonucleotides may

be synthesized on an automated nucleic acid synthesizer. Alternatively, the oligonucleotides may be synthesized enzymatically through the use of flanking or primer sequences at the 5' and 3' ends. In another alternative, the oligonucleotides may be synthesized by solution phase chemistry. It is to be understood, however, that the scope of the present invention is not to be limited to any particular means of synthesis.

The oligonucleotides of the present invention may be administered in conjunction with an acceptable pharmaceutical carrier as a pharmaceutical composition. Such pharmaceutical compositions may contain suitable excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Such oligonucleotides may be administered by intramuscular, intraperitoneal, intraveneous, or subdermal injection in a suitable solution. Preferably, the preparations, particularly those which can be administered orally and which can be used for the preferred type of administration, such as tablets, dragees and capsules, and preparations which can be administered rectally, such as suppositories, as well as suitable solutions for administration parenterally or orally, and compositions which can be administered buccally or sublingually, including inclusion compounds, contain from about 0.1 to 99 percent by weight of active ingredients, together with the excipient. It is also contemplated that the oligonucleotides may be administered topically.

The pharmaceutical preparations of the present invention are manufactured in a manner which is itself well known in the art. For example, the pharmaceutical preparations may b made by means of conventional mixing, granulating, dragee-

making, dissolving or lyophilizing processes. The process to be used will depend ultimately on the physical properties of the active ingredient used.

Suitable excipients are, in particular, fillers such as sugar, for example, lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example, tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch or paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added, such as the above-mentioned starches as well as carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are flow-regulating agents and lubricants, such as, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragee cores may be provided with suitable coatings which, if desired, may be resistant to gastric juices. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate, are used. Dyestuffs and pigments may be added to the tablets of dragee coatings, for example, for identification or in order to characterize different combinations of active compound doses.

Other pharmaceutical preparations which can be used orally include push-fit capsules mad of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the oligonucleotide in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols, or higher alkanols. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble or water-dispersible form. In addition, suspensions of the active compounds as appropriate oil injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethyl

cellulose, sorbitol and/or dextran. Optionally, th suspension may also contain stabilizers.

Additionally, the compounds of the present invention may also be administered encapsulated in liposomes, wherein the active ingredient is contained either dispersed or variously present in corpuscles consisting of aqueous concentric layers adherent to lipidic layers. The active ingredient, depending upon its solubility, may be present both in the aqueous layer, in the lipidic layer, or in what is generally termed a liposomic suspension. The hydrophobic layer, generally but not exclusively, comprises phospholipids such as lecithin and sphingomycelin, steroids such as cholesterol, surfactants such as dicetylphosphate, stearylamine, or phosphatidic acid, and/or other materials of a hydrophobic nature. The diameters of the liposomes generally range from about 15 nm to about 5 microns.

The oligonucleotides are administered to a host, such as a human, in an amount effective to inhibit the IFN-γ function. Thus, the oligonucleotides may be used prophylactically or therapeutically. Preferably, the oligonucleotides are administered to a host so as to provide a concentration of oligonucleotide in the blood of from about 10 nanomolar to about 500 micromolar, preferably from about 5 micromolar to about 100 micromolar. It is also contemplated that the oligonucleotides may be administered *in vitro* or *ex vivo* as well as *in vivo*.

The oligonucleotides of the present invention may also be employed as diagnostic probes for determining the presence of IFN-γ, and thereby determining the need for modulation of its function or activity. In such embodiments, a modified or

unmodified oligonucleotide of the present invention is added to a sample suspected of containing IFN-γ. The oligonucleotide may be labeled with a detectable marker, including but not limited to, a radioisotope, a biotin moiety, a chromophore, a fluorescent moiety, or an enzyme label. Thus, the oligonucleotide may be employed in a variety of assay methods for the detection of IFN-γ, such methods including sandwich assays, competitive assays, ELISA, inhibition assays, and other assays known to those skilled in the art.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

The following examples illustrate oligonucleotide binding to IFN-γ and inhibition of IFN-γ binding with its receptor protein.

Example 1

Oligonucleotide Binding to IFN-γ

In order to demonstrate that oligonucleotides were capable of binding tightly to IFN-γ they were synthesized on an oligonucleotide synthesizer to have a specified sequence. In the case of RNA oligonucleotides, the oligonucleotide either was synthesized directly as RNA or else the corresponding DNA sequence was synthesized and the RNA to be tested was generated by *in vitro* transcription of the DNA using a mixture of nucleoside triphosphates and T7 RNA

polymerase. The synthetic oligonucleotides were ^{32}p-labelled either at the 5' end using γ^{32}p-ATP and T4 polynucleotide kinase or internally using (α^{32}p-NTPs in the transcription reaction. Portions of the labelled oligonucleotide being tested were incubated with various concentrations of IFN-γ in a binding buffer of 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 3mM $MgCl_2$ for about 20 minutes at ambient temperature (ca. 23°C). The labelled oligonucleotide bound to IFN-γ protein was recovered by filtration of the binding reaction through a nitrocellulose filter. After washing the filter with excess binding buffer, the radioactive oligonucleotide retained by the filter was determined by scintillation counting and the percentage of the counts retained was plotted against the concentration of protein present in the binding reaction. Figure 1 presents representative results for oligonucleotides of the invention (SEQ ID NOS: 3, 8 AND 26). Also shown in Figure 1 is the much weaker binding of a mixture of 98 base random-sequence RNA to IFN-γ.

Example 2

Oligonucleotides inhibit binding of IFN-γ with IFN-γ receptor

Competition assays were performed to demonstrate that binding of RNA oligonucleotides to IFN-γ blocks the interaction with IFN-γ receptor. In the first competition assay, microtiter wells of a 96-well ELISA plate were coated with 20 pmols of IFN-γ in 50 μl of 150 mM Na_2CO_3, 35 mM $NaHCO_3$ pH 9.6 for about 16 hours at 4°C. Various amounts of oligonucleotide (12 or 60 pmols) were added to different wells in 50 μl binding buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 3 mM $MgCl_2$) and incubated for about 5 minutes at room temperature (ca. 23°C) to allow for binding of the

oligonucleotide and the IFN-γ. After incubation, 6 pmols of IFN-γ receptor protein were added in 50 μl binding buffer and the solution was incubated for at least 120 minutes at 37°C. The IFN-γ receptor used for this assay was a recombinant soluble fusion protein consisting of the extracellular domain of the human IFN-γ receptor at the amino end of the protein fused to a mouse kappa light chain immunoglobulin at the carboxyl terminus. After washing with binding buffer, the amount of receptor bound to IFN-γ was determined by adding an enzyme-linked antibody against the mouse kappa light chain portion of the fusion protein [1:500 dilution of antibody (Caltag Laboratories) in phosphatebuffered saline containing 1 mg/ml bovine serum albumin] for 60 minutes at 37°C followed by a chromogenic substrate for the linked enzyme [H_2O_2/ABTS (Boehringer Mannheim)], and incubation for an additional 30 minutes at room temperature. The anti-kappa chain antibody does not interfere with the binding of the IFN-γ to its receptor. A reduction in the amount of receptor bound to IFN-γ in the presence of test oligonucleotide indicated that the oligonucleotide blocked the interaction of the cytokine with its receptor. All tested oligonucleotides of the instant invention blocked binding of IFN-γ to its receptor. In contrast a pool of random-sequence oligonucleotides had a minimal effect on the receptor binding. Figure 2 presents representative results of assays testing the inhibition by oligonucleotides SEQ ID NOS: 2 and 3.

A second competition assay was also performed to demonstrate the ability of oligonucleotides to inhibit IFN-γ binding to its receptor. In this assay, wells of the microtiter plate were coated with 8 pmols of the recombinant IFN-γ receptor fusion protein described above (in 50 μl phosphate-buffered saline for about 16 hours at 4°C). IFN-γ

(2 pmols) was incubated with various amounts of oligonucleotide in 50 μl binding buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 3mM $MgCl_2$) for 5 minutes at room temperature and then the solutions were added to wells containing the receptor protein (final volume 100 μl) to allow binding of receptor and ligand. After washing, the amount of IFN-γ bound to receptor was determined using non-neutralizing anti-IFN-γ antibody (Biosource International) in an enzyme-linked detection assay (Horseradish peroxidase-coupled goat anti-mouse Fc antibody, Accurate Chemical and Scientific Corp.). Reduction of IFN-γ binding in the presence of oligonucleotide indicated inhibition of the receptor-ligand interaction by the oligonucleotides. Figure 3 presents representative results for the second assay with three oligonucleotides of the instant invention (SEQ ID NOS: 2, 3, and 11) and a mixture of 98 base random sequence RNA for comparison. As is evident from these results, the oligonucleotides of the instant invention are more effective at inhibiting the interaction of IFN-γ and its receptor than the mixture of random-sequence RNA.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT(S): Coppola, Georg R.
Beutel, Bruc A.
Bertelsen, Arthur H.

(ii) TITLE OF INVENTION: Inhibition of Interferon-γ with Oligonucleotides

(iii) NUMBER OF SEQUENCES: 39

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein

(B) STREET: 6 Becker Farm Road

(C) CITY: Roseland

(D) STATE: New Jersey

(E) COUNTRY: USA

(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 inch diskette

(B) COMPUTER: IBM

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WordPerfect 5.1

(vi) CURRENT APPLICATION DATA

(A) APPLICATION NUMBER: Unassigned

(B) FILING DATE: Unassigned

(C) CLASSIFICATION: Unassigned

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Herron, Charles J.

(B) REGISTRATION NUMBER: 28,019
(C) REFERENCE/DOCKET NUMBER: 23550-114

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO: 1
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 97 BASES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(iii) HYPOTHETICAL: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

GGGAGAAGUA GUGUAGGAAU UCGAGCAAGA AGUCCCUGAG GAGUGAUGUC 50
AUUCCUGCCC CAUGAUAUGG AGACUUCUUA ACUCGAGAGG UCACAGU 97

(3) INFORMATION FOR SEQ ID NO: 2
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 98 BASES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(iii) HYPOTHETICAL: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

GGGAGAAGUA GUGUAGGAAU UCAUUUAAGG GACCUUCUUG CACUUGGAUA 50
GCCUUUCUUG AGGGGCCAGU UCAUCUCUCC AGCUCGAGAG GUCACAGU 98

(4) INFORMATION FOR SEQ ID NO: 3
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 98 BASES
(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

GGGAGAAGUA GUGUAGGAAU UCUAAGUUGA GGCUCGUACU UGUCCUUUGA 50

UUUUCUGUGU GGGAUGUUAA UAUUCGUGUG GUCUCGAGAG GUCACAGU 98

(5) INFORMATION FOR SEQ ID NO: 4

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 97 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

GGGAGAAGUA GUGUAGGAAU UCUUGUUCCU AGUCCUAGCG AAAUUGUUGA 50

UUAAAAAGUG GUGCUCGUCC UAACUCGAUA UCUCGAGAGG UCACAGU 97

(6) INFORMATION FOR SEQ ID NO: 5

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5

GGGAGAAGUA GUGUAGGAAU UCACAAGGAC CCCUGCUUUC CCGGACCUCA 50

UUCCCAUAAG UUGUAUGGCG GGAGUUUCUG GACUCGAGAG GUCACAGU 98

(7) INFORMATION FOR SEQ ID NO: 6

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

GGGAGAAGUA GUGUAGGAAU UCUAUUGCCU CAGAGAGACU CAACGUCACU 50
GGAAUGCCAA UCUAUACAUG UUGGUUUUCG CCCUCGAGAG GUCACAGU 98

(8) INFORMATION FOR SEQ ID NO: 7

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7

GGGAGAAGUA GUGUAGGAAU UCUGUCUCCA GUACCUCUGA UUCUAUAAAA 50
GUCUUACGUA GAAUGCCCUC GAUAAUUUAU AUCUCGAGAG GUCACAGU 98

(9) INFORMATION FOR SEQ ID NO: 8

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8

GGGAGAAGUA GUGUAGGAAU UCUAAGUUGA GGCUCGUAUU UCGCCGGAUC 50
GUCUUAGAGU AUAAGAGACU UGGUCUAUUC ACCUCGAGAG GUCACAGU 98

(10) INFORMATION FOR SEQ ID NO: 9

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9

GGGAGAAGUA GUGUAGGAAU UCCCGCAGUG AUUAAAUAUG CAUGGCCAAC 50
UACCUAGGUG UGUAUCUCUC UCCCCUACCU CGCUCGAGAG GUCACAGU 98

(11) INFORMATION FOR SEQ ID NO: 10

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10

GGGAGAAGUA GUGUAGGAAU UCUUUUAGAC UGUUCCGGUU UUCUAAACCG 50
UGAGGGGUAU ACUCUUAACC CUUGGUGUAU GCCUCGAGAG GUCACAGU 98

(12) INFORMATION FOR SEQ ID NO: 11

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 97 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11

GGGAGAAGUA GUGUAGGAAU UCCUUUUGUG ACCGCUGGGC ACACAACCGU 50
AGCCACCCUC GAAUUAGCGA CUGCCCGUCG UCUCGAGAGG UCACAGU 97

(13) INFORMATION FOR SEQ ID NO: 12

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 97 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12

GGGAGAAGUA GUGUAGGAAU UCUCGCCGGA CCGCUGGACC UGACAGGGUC 50
ACCCUCGUGC UGCAUAAACC CCCUUUUCUA GCUCGAGAGG UCACAGU 97

(14) INFORMATION FOR SEQ ID NO: 13

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13

GGGAGAAGUA GUGUAGGAAU UCUGAUUCUU GAUGGUAGUG UGAGAUAGAA 50
CGCUCCUUAU CACGCCUACU AUGUGUACCU UCCUCGAGAG GUCACAGU 98

(15) INFORMATION FOR SEQ ID NO: 14

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14

GGGAGAAGUA GUGUAGGAAU UCACCUACCG GUGUCUCAGC CACCCAACUC 50
UUAAAAAGGG AAGUGCUGUC CUCGGCCUUU AGCUCGAGAG GUCACAGU 98

(16) INFORMATION FOR SEQ ID NO: 15

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 97 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15

GGGAGAAGUA GUGUAGGAAU UCUAAGGUGA GGCUCGGUCU CUUCUCGUCA 50
UGUAUAUAGC ACAAGUUGCC GUCGUUUACU CCUCGAGAGG UCACAGU 97

(17) INFORMATION FOR SEQ ID NO: 16

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16

GGGAGAAGUA GUGUAGGAAU UCAUUGUGAG CUCUGGGUAG UAUUUACUGC 50
CACCCUCUGU AGUUGGAUUC CUGUCGAGUU UUCUCGAGAG GUCACAGU 98

(18) INFORMATION FOR SEQ ID NO: 17

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 97 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17

GGGAGAAGUA GUGUAGGAAU UCAUAUUAAU AUCGACCAUC GUAUUUCACU 50
UCUCAGUCCG GAGUCGUACG CAGCAUAUUG ACUCGAGAGG UCACAGU 97

(19) INFORMATION FOR SEQ ID NO: 18

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 97 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18

GGGAGAAGUA GUGUAGGAAU UCACGUAAAA GAAUAUGCUG GCGGUGCGGG 50
UACGAAUAUU CAUGUGAGCA GUUGGACUGU ACUCGAGAGG UCACAGU 97

(20) INFORMATION FOR SEQ ID NO: 19

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19

GGGAGAAGUA GUGUAGGAAU UCAUUAUUGG CCUUGAUAUU AUAUAGCUUC 50
GCUACACUUG GGCGCAAGGU AUUGCCGUUC GUCUCGAGAG GUCACAGU 98

(21) INFORMATION FOR SEQ ID NO: 20

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 96 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GGGAGAAGUA GUGUAGGAAU UCUACGCUAU CUUUAUAAGU UUGGAUUGUC 50
CUAAGCUAAU UUUUAUCAUA GGAGGGGCUA CUCGAGAGGU CACAGU 96

(22) INFORMATION FOR SEQ ID NO: 21

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GGGAGAAGUA GUGUAGGAAU UCAGUGCUUA CCGGCCCAGU AACCUAUUGA 50
CGAAGCGUUC CGAGGUAAUC CCGCAUGUAA ACCUCGAGAG GUCACAGU 98

(23) INFORMATION FOR SEQ ID NO: 22

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GGGAGAAGUA GUGUAGGAAU UCAACACGCC GGGAACUUAC UUUGACGUUG 50
CGAUCCCCCA AUUAUAAGAC UAUCUGGAUC ACCUCGAGAG GUCACAGU 98

(24) INFORMATION FOR SEQ ID NO: 23

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GGGAGAAGUA GUGUAGGAAU UCUAAGUUGA AGCUCACUAC UGCGUACAUU 50
GAGAGAUUGA CAAUUUCAAU GCGAUGGUUU GCCUCGAGAG GUCACAGU 98

(25) INFORMATION FOR SEQ ID NO: 24

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GGGAGAAGUA GUGUAGGAAU UCCUCUCGAA UUACCUACAG UUCACCCCUC 50
UAUGUAAGUG AUUCCUCUUC AAACUACUUC CUCUCGAGAG GUCACAGU 98

(26) INFORMATION FOR SEQ ID NO: 25

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25

GGGAGAAGUA GUGUAGGAAU UCUGAUGCUU UGUUAAAAGC ACGAUAAGUU 50
GAGGCUCGAC UGGGACUCUA AUUUUACCUC CGCUCGAGAG GUCACAGU 98

(27) INFORMATION FOR SEQ ID NO: 26

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26

GGGAGAAGUA GUGUAGGAAU UCUAAGUUGA CGCUCGCCAA UUCCUCCCGU 50
UCUGAGGGUA AAACGCAUAC UCGUGUUAGG CUCUCGAGAG GUCACAGU 98

(28) INFORMATION FOR SEQ ID NO: 27

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27

GGGAGAAGUA GUGUAGGAAU UCCUCACCUU CAAUGCGCUC CAAAACACCU 50
CUGGGUCAUG CAUGGACCAC CCUCGGGAAA UUCUCGAGAG GUCACAGU 98

(29) INFORMATION FOR SEQ ID NO: 28

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 98 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28

GGGAGAAGUA GUGUAGGAAU UCUAAGUUGA UGCUGACUAU GGAUUCCAGG 50
CUUUCUGCGU AUCAUCGUCU AUGUUUUCUA GUCUCGAGAG GUCACAGU 98

(30) INFORMATION FOR SEQ ID NO: 29

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 97 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29

GGGAGAAGUA GUGUAGGAAU UCUAGACUGC UCUGUUCGAA UUUCCCAGUG 50
UAAAUCAGGU CUUAAUUUCG UGUAACAAGU ACUCGAGAGG UCACAGU 97

(31) INFORMATION FOR SEQ ID NO: 30

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30

AAGUUG 6

(32) INFORMATION FOR SEQ ID NO: 31

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 8 BASES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(iii) HYPOTHETICAL: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31

UGANGCUC 8

(33) INFORMATION FOR SEQ ID NO: 32
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 14 BASES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(iii) HYPOTHETICAL: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32

UAAGUUGANG CUCG 14

(34) INFORMATION FOR SEQ ID NO: 33
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 7 BASES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(iii) HYPOTHETICAL: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33

GCACCNC 7

(35) INFORMATION FOR SEQ ID NO: 34
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 10 BASES
(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34

GCCACCCUCG 10

(36) INFORMATION FOR SEQ ID NO: 35

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 93 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35

AAGUAGUGUA GGAAUUCUAA GUUGAGGCUC GUACUUGUCC UUUGAUUUUC 50
UGUGUGGGAU GUUAAUAUUC GUGUGGUCUC GAGAGGUCAC AGU 93

(37) INFORMATION FOR SEQ ID NO: 36

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 43 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36

AAGUAGUGUA GGAAUUCUAA GUUGAGGCUC GUACUUGUCC UUU 43

(38) INFORMATION FOR SEQ ID NO: 37

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 60 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37

GGGAGAAGUA GUGUAGGAAU UCUAAGUUGA GGCUCGUACU UGUCCUUUGA 50
UUUUCUGUGU 60

(39) INFORMATION FOR SEQ ID NO: 38

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 48 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38

GGGAGAAGUA GUGUAGGAAU UCUAAGUUGA GGCUCGUACU UGUCCUUU 48

(40) INFORMATION FOR SEQ ID NO: 39

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 40 BASES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39

GGGAGAAGUA GUGUAGGAAU UCUAAGUUGA GGCUCGUACU 40

What Is Claimed Is:

1. An oligonucleotide that binds to IFN-γ upon contact therewith.

2. The oligonucleotide of claim 1 that modulates th activity of IFN-γ upon binding therewith.

3. The oligonucleotide of claim 1 that binds with the target IFN-γ at a K_d of not greater than about 30 nM.

4. The oligonucleotide of claim 1 comprising at least one sequence selected from the group consisting of SEQ ID NOS: 1 through 39.

5. The oligonucleotide of claim 3 comprising at least one sequence selected from the group consisting of SEQ ID NOS: 30 to 34.

6. A method of inhibiting IFN-γ function which comprises contacting IFN-γ with an inhibitory amount of an oligonucleotide which inhibits IFN-γ function.

7. The method of claim 6 in which the oligonucleotide comprises at least one sequence selected from the group consisting of SEQ ID NOS: 1 through 39.

8. The method of claim 6 in which the oligonucleotide comprises at least one sequence selected from the group consisting of SEQ ID NOS: 30 through 34.

9. The method of claim 6 which comprises inhibiting IFN-γ function in an individual in need thereof by

administering to the individual an IFN-γ inhibitory amount of an oligonucleotide which inhibits IFN-γ function.

10. A method of detecting th presence of IFN-γ in a sample by contacting the same with an oligonucleotide that binds to IFN-γ, removing the unbound oligonucleotide and measuring the amount of oligonucleotide that remains bound to components in the sample.

11. The method of claim 8 in which the oligonucleotide comprises at least one sequence selected from the group consisting of SEQ ID NOS: 1 through 39.

12. The method of claim 8 in which the oligonucleotide comprises at least one sequence selected from the group consisting of SEQ ID NOS: 30 through 34.

Figure 1.

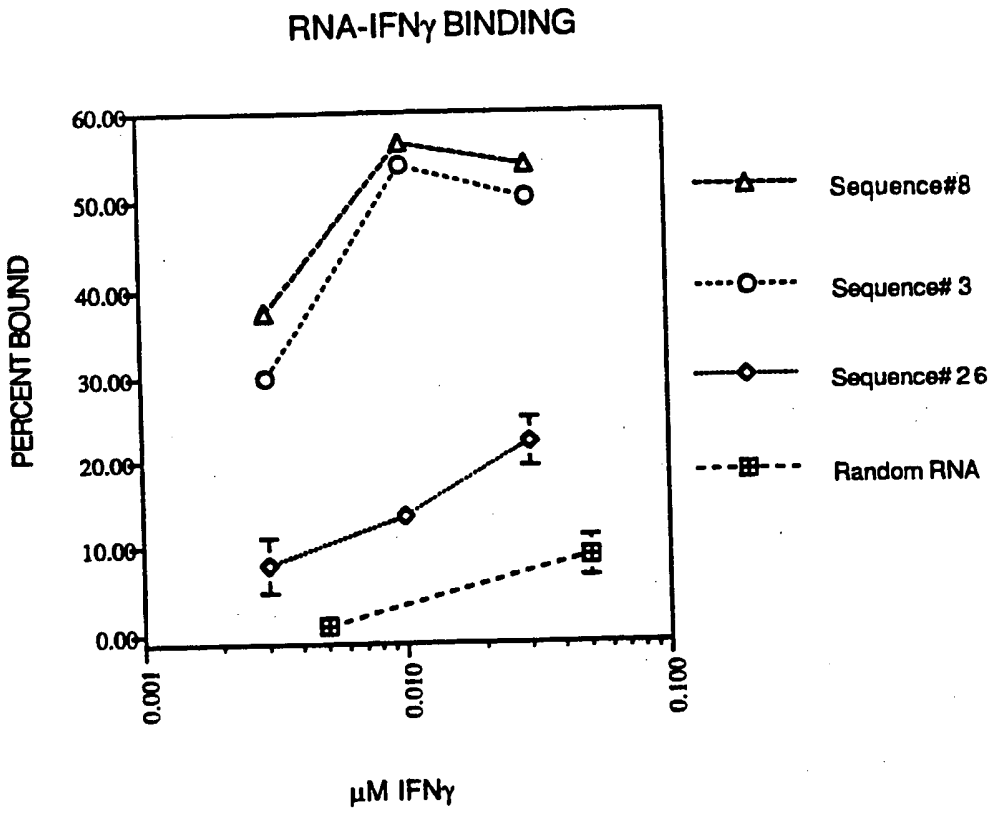

RNA-IFNγ BINDING
PERCENT BOUND
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0.001
0.010
0.100
μM IFNγ
Sequence#8
Sequence# 3
Sequence# 26
Random RNA

Figure 2.

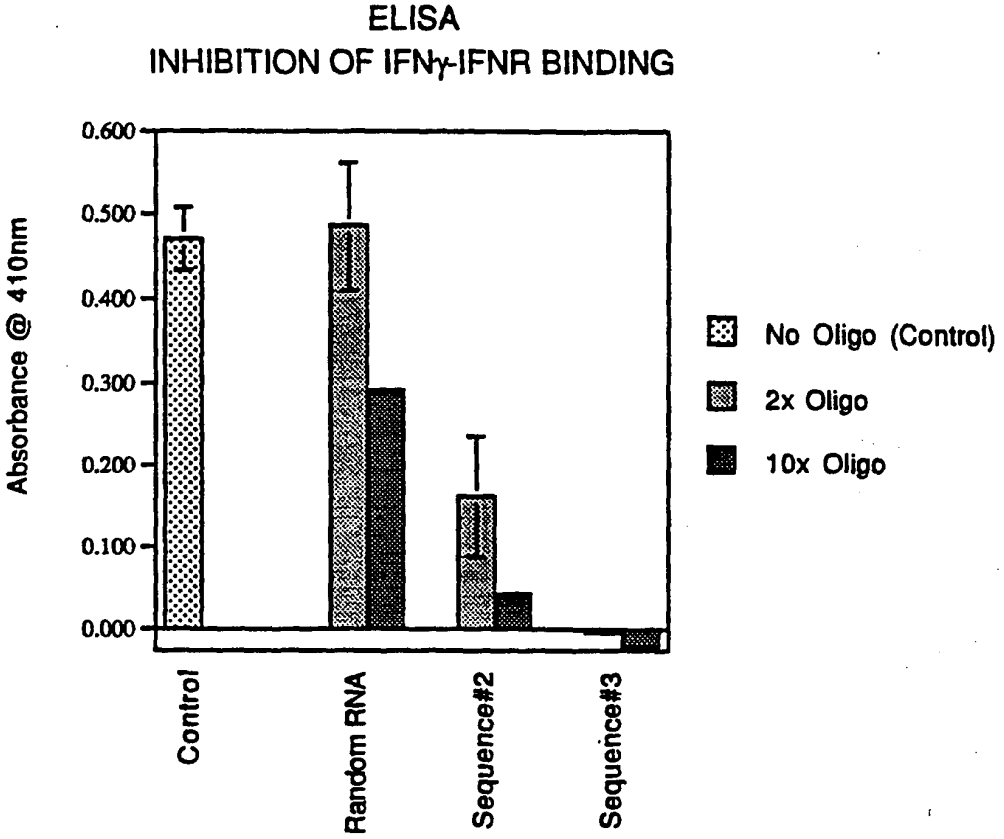
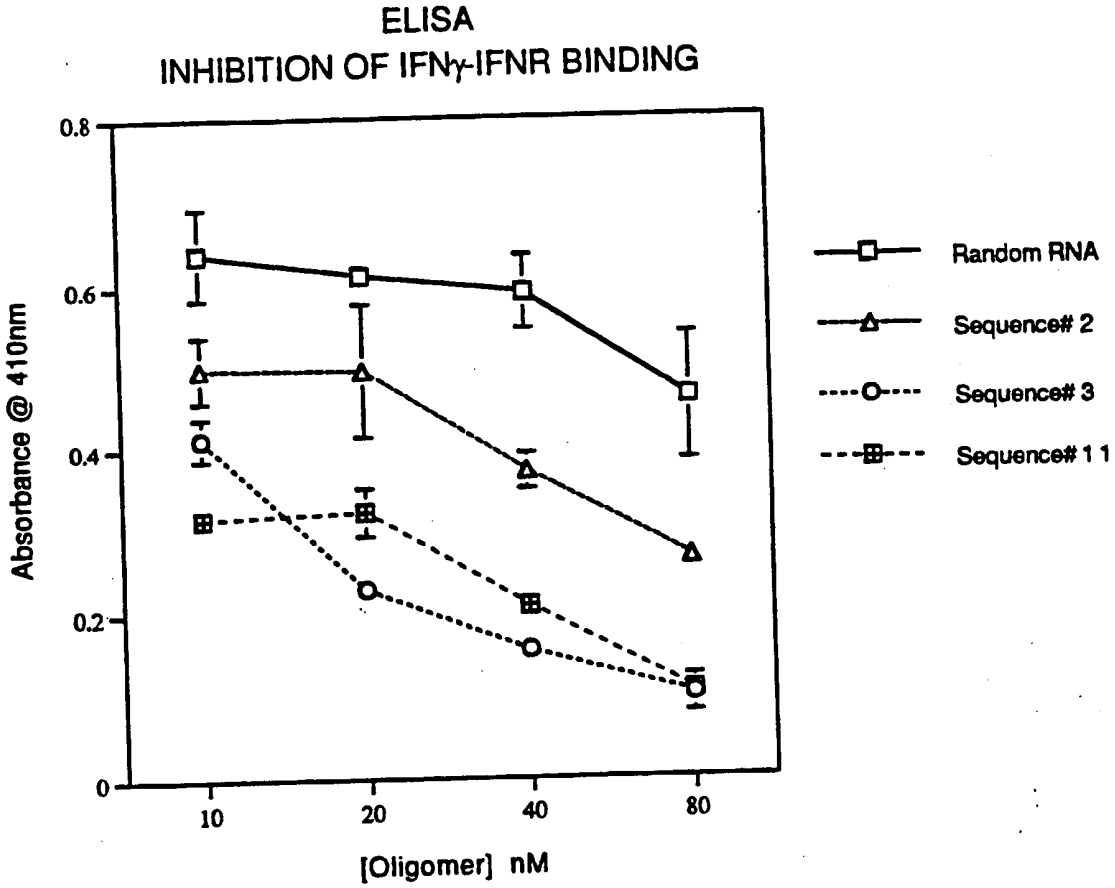

ELISA
INHIBITION OF IFNγ-IFNR BINDING
Absorbance @ 410nm
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Control
Random RNA
Sequence#2
Sequence#3
No Oligo (Control)
2x Oligo
10x Oligo

Figure 3.


ELISA
INHIBITION OF IFNγ-IFNR BINDING
Absorbance @ 410nm
0.8
0.6
0.4
0.2
0
10
20
40
80
[Oligomer] nM
Random RNA
Sequence# 2
Sequence# 3
Sequence# 1 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06897

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07H 21/02, 21/04; C12Q 1/68; G01N 33/50
US CL :435/6, 7.1; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, MEDLINE, BIOSIS, WPI
search terms: aptamer, interferon gamma, oligonucleotide, polynucleotide, inhibit, bind

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,681,931 (OBERMEIER ET AL) 21 July 1987, col. 1, lines 46-50.	1-9
Y	Nucleic Acids Research, Volume 21, No. 18, issued 1993, Crameri et al, "10^{20}-Fold aptamer library amplification without gel purification", page 4410, see entire document.	1-9
Y	FASEB Journal, Volume 7, issued January 1993, J. M. Burke et al, "In vitro selection and evolution of RNA: applications for catalytic RNA, molecular recognition, and drug discovery", pages 106-112, especially pages 110-111.	1-9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 SEPTEMBER 1994	SEP 29 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID SCHREIBER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06897

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	Journal of Biological Chemistry, Volume 268, No. 24, issued 25 August 1993, Padmanabhan et al, "The Structure of α-Thrombin Inhibited by a 15-Mer Single-stranded DNA Aptamer", pages 17651-17654, especially page 17653, first full paragraph.	1-9
Y	Nature, Volume 355, issued 06 February 1992, L. C. Bock et al, "Selection of single-stranded DNA molecules that bind and inhibit human thrombin", pages 564-566, especially Table 1.	1-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/06897

Box I Observations where certain claims were f und unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9

Remark n Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I.Claims 1-9, drawn to an oligonucleotide that binds to IFN-γ and modulates the activity of the protein and a method of inhibiting IFN-γ function using an inhibitory amount of said oligonucleotide, classified in Class 536/23.1, for example.
II.Claims 10-12, drawn to a method of detecting the presence of IFN-γ in a sample, classified in Class 435/6, for example.
Detailed Reasons for Holding Lack of Unity of Invention:
PCT Rule 13.1 recites the basic principle of unity of invention that an application should relate to only one invention or, if there is more than one invention, that applicant would have a right to include in a single application only those inventions which are so linked as to form a single general inventive concept. According to PCT Rule 13.2, a group of inventions is linked to form a single inventive concept where there is a technical relationship among the inventions that involves at least one common or corresponding special technical feature that defines the contribution which each claimed invention, considered as a whole, makes over the prior art.
The two inventions of this application consist of: 1) an oligonucleotide that binds to IFN-γ and modulates the activity of the protein and a method of inhibiting IFN-γ function using an inhibitory amount of said oligonucleotide and 2) a method of detecting the presence of IFN-γ in a sample. The two inventions are not linked by a special technical feature within the meaning of PCT Rule 13.2 because binding of nucleic acids to interferons is well known in the art.